Molecular Breeding **4:** 359–367, 1998.

Molecular markers for resistance to *Heterodera glycines* in advanced soybean germplasm

J.A. Heer[1], H.T. Knap[1,*], R. Mahalingam[1], E.R. Shipe[1], P.R. Arelli[2] and B.F. Matthews[3]
[1]*Department of Agronomy, Clemson University, Box 340359, Poole Agriculture Center, Clemson, SC 29634, USA (*author for correspondence; e-mail hskrpsk@clemson.edu);* [2]*Department of Agronomy, University of Missouri-Columbia, 117 Curtis Hall, Columbia, MO 65211, USA;* [3]*USDA/ARS, Soybean and Alfalfa Research Laboratory, Beltsville, MD 20705, USA*

Received 4 June 1997; accepted in revised form 9 April 1998

Key words: advanced germplasm, markers, soybean cyst nematode

Abstract

Germplasm line J87-233 is resistant to soybean cyst nematode (SCN) races 1, 2, 3, 5 and moderately resistant to race 14 with resistance derived from 3 primitive sources, 'Peking', PI 88788 and PI 90763. $F_{2:3}$ progeny of J87-233 and SCN-susceptible 'Hutcheson' cross were evaluated for response to SCN races 1, 2, 3, 5 and 14. Linkage groups (LG) A, B, F, G, J, M, N, S were tested with 215 genomic clones and 45 decamers for parental genotypes. QTL for race 1 and QTL for race 3 were detected on LG A2, the region of BLT65V and SCAR $548/563_{1100/1025,975.}$ The cluster analysis of 12 soybean cultivars and 38 plant introductions confirmed association of $SCAR_{1100/1025,975}$ with resistance to races 1 and 3, and suggested possible DNA rearrangements that might give rise to new resistance specificities in the region. The highly significant association of K69T marker with SCN race 1 resistance in conjunction with its location, 18.5 cM from the reported QTL, exemplifies the importance of the QTL locus on LG G and suggests expansion of the linkage map in the LG G-terminal region. Detected interaction between loci on LG A2 and LG G, and also with loci on LG F and LG M, may play a significant role in the genotype-specific response to SCN. Identification of two major regions on LG A2 and LG G for SCN resistance shows their applicability to advanced germplasm, however, transmission of molecular marker alleles indicates that applied markers are not yet reliable in revealing all possible recombination events in breeding for SCN resistance.

Introduction

One of the most destructive pests of soybean (*Glycine max* (L.) Merrill) in the USA is soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe. In efforts to combat this pathogen, soybean breeders have developed cultivars with genetic resistance derived from primitive plant introductions (PIs). Resistance to SCN is a multigenic trait, such that PIs may express resistance through mechanisms conferred by several different genes or alleles [14]. Breeding efforts have resulted in cultivars that have inherited resistance genes from one or two resistant PIs. This can be described as 'narrow-based resistance' as compared to 'broad-based resistance' where several PIs of diverse origins are the contributors of resistance to a cultivars' genetic profile. Cultivars with broad based resistance may impart a stronger buffering capacity to the challenges of race shifts within nematode field populations, and can exhibit resistance to multiple SCN races.

Previous efforts to develop a comprehensive genetic linkage map and establish useful molecular marker associations with SCN resistance genes have most often utilized populations created from wide crosses involving a PI and an advanced susceptible cultivar [5, 6, 12, 21]. Linkage groups (LGs) A2 and G [18] have been established as the likely locations of major QTLs for SCN resistance, with minor QTLs found on linkage groups F, J, K, M and N [5, 6, 12, 21]. In this study we applied the cumulative SCN resistance QTL mapping information to a population derived from advanced breeding lines of

complex genetic backgrounds. Germplasm line J87-233 has inherited SCN resistance from three resistance sources, 'Peking', PI 88788 and PI 90763 [23]. The pedigree for J87-233 is given in Figure 1. This line was chosen for our study because it represents genotypes more likely to be used in an actual breeding program for SCN resistance than a single PI. J87-233 was crossed with SCN-susceptible cultivar 'Hutcheson', another advanced genotype [3]. We refer to this cross as an 'authentic' breeding cross which could be used to develop a high-yielding cultivar with broad-based SCN resistance within a few generations of selection. We characterized the specific chromosomal regions that have persisted throughout selection for SCN resistance in the development of J87-233, and evaluated the effectiveness of previously established SCN resistance molecular markers, as applied to this authentic breeding population.

Materials and methods

Plant material

A soybean population of 125 F_2 individuals was developed from a cross between germplasm J87-233 and cv. 'Hutcheson' at Clemson University. J87-233 is of maturity group V and has tawny pubescence and purple flowers. It was released in 1992 by the USDA-ARS because of its resistance to SCN races 1, 2, 3, 5 and moderate resistance to race 14, as well as resistance to root-knot nematode, *Meloidogyne incognita* [23]. The primitive cultivar 'Peking', PI 88788, and PI 90763 contributed SCN resistance to germplasm J87-233. 'Hutcheson' is a SCN-susceptible cultivar of maturity group V with gray pubescence and white flower color [3]. It is widely grown in the southern USA because of its superior agronomic qualities and high seed yield. The $F_{2:3}$ progeny of the J87-233 $\times$ 'Hutcheson' cross as well as 'Peking', PI 88788 and PI 90763 were used in all molecular analyses. Moreover, 19 soybean cultivars and 31 PIs screened for SCN resistance were used in molecular analysis involving SCAR 548/563.

DNA isolation

Soybean DNA was extracted from first and second trifoliolate leaves of greenhouse plants using the CTAB method by Bendich and Rogers [2] with modifications for soybean by Keim *et al.* [7].

RFLP analysis

Restriction fragment length polymorphism analyses were performed with DNA of parental and progeny genotypes as described by Skorupska *et al.* [19]. Five restriction enzymes were used: *Eco*RI, *Eco*RV, *Dra*I, *Taq*I, and *Hin*dIII (Promega, Madison, WI). Soybean genomic clones were obtained from Biogenetic Services, Inc. (Brookings, SD). Polymorphisms detected between parents were applied to the $F_{2:3}$ progeny. Markers were anchored to linkage groups using the same probe/enzyme combinations that had been used to develop the soybean molecular linkage map reported by Shoemaker and Specht [18].

RAPD analysis

The RAPD analyses were performed with procedures described by Skorupska *et al.* [19]. Primers used in RAPD analyses were obtained from Operon Technologies (Alameda, CA).

The SCAR primers 548 and 563 (5′-GCAGATATC CAACAGTTGGGAC-3′ and 5′-TGGAATGACTGCA ACCTGAGAG-3′, respectively) were developed from the sequence of a 1 kb fragment of the aspartokinase-homoserine dehydrogenase gene (AK-HSDH) which has been mapped near the gene conferring seed coat color, *i* locus, on LG A of the soybean molecular map [22]. This region of LG A has been reported to contain QTL for SCN resistance [11, 21]. The PCR reaction for this primer set required d-NTPs (Pharmacia Biotech, Piscataway, NJ) at 240 μM, 4 mM $MgCl_2$, 50 mM KCl, 10 mM Tris pH 8.0, 1.25 units Ampli-*Taq* polymerase (Perkin-Elmer, Norwalk, CT), 0.12 ng primer and ca. 10 ng DNA. The PCR thermal profile was 34 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 1.5 min. Samples were then subjected to electrophoresis in a 2% TBE agarose gel.

Bioassay

Soybean cyst nematode bioassays were performed on J87-233, 'Hutcheson', PI 88788, PI 90763, 'Peking' and the 125 $F_{2:3}$ progeny. Ten replications per F_2 genotype were analyzed for each of the five SCN races (1, 2, 3, 5 and 14). Including resistance sources and parental lines, total of 6500 seedlings were tested. Soybean cyst nematode race 1, 2, 3, 5 and 14 populations were developed, maintained and used in bioassays as described in Rao-Arelli and Anand [13] and

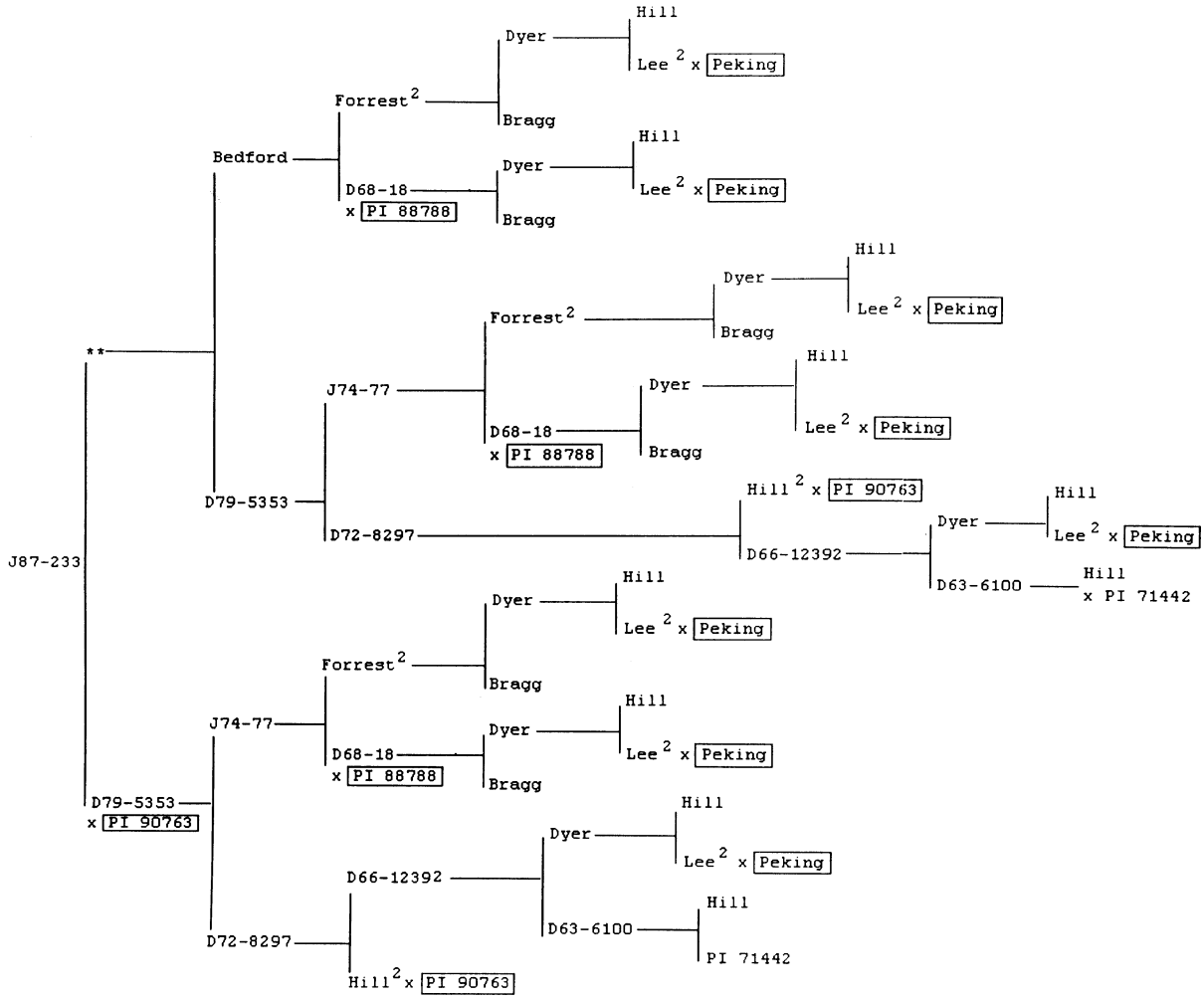

Figure 1. Pedigree Diagram of Germplasm J87-233. Resistant sources 'Peking', PI 88788 and PI 90763 are in boxes.

Arelli and Wilcox [1]. SCN race identity tests were performed as described in Riggs and Schmitt [14].

Indices of parasitism (IP) were calculated using bioassay cyst counts for each genotype. The average number of cysts per genotype was calculated and expressed as a percentage of the average number of cysts found on the susceptible parent, 'Hutcheson'.

Data analysis

A linear regression of RFLP and RAPD scores against IP results for each of the five SCN races was conducted using SAS, version 6.0 [16]. Goodness of fit for marker segregation was tested using chi-square analysis. Interactions between unlinked markers detecting significant loci for SCN resistance were evaluated by two-way ANOVA. Linkage analyses were conducted using MAPMAKER/EXP version 3.0 [9] using a minimum LOD score of 3.0 and maximum distance of 30 cM. Interval mapping was conducted in QTL regions using MAPMAKER/QTL version 1.1 [10].

NTSYS-pc version 1.80 [15] was used to construct a consensus tree by unweighted pair group method, arithmetic average (UPGMA) for 50 soybean genotypes based on their responses to SCN races 1 and 3. The SIMQUAL (similarity for qualitative data) function of this program computed distance association coefficients for the binary RFLP data using the matching (SM) equation m/n, where m is the number of loci for which two genotypes carry the same allele, and n is the total number of loci described. The SCAR molecular data for the 50 genotypes was juxtaposed to the tree for comparison. The majority-rule consen-

sus tree was determined using the SAHN (Sequential, Agglomerative, Hierarchical, and Nested clustering) function of NTSYS-pc [15].

Results

Molecular Polymorphism

A total of 215 soybean genomic clones were hybridized to digested genomic DNA of SCN-resistant J87-233 and SCN-susceptible 'Hutcheson'. Most of these clones were on linkage groups for which SCN response-marker associations have been reported. Polymorphism between J87-233 and 'Hutcheson' was detected by 83 probes (39%) with at least one restriction enzyme. Overall, 33% of the clones that were polymorphic for the J87-233 × 'Hutcheson' cross (ca. 11% of the total genomic clones tested) corresponded to loci previously assigned to eight LGs of the USDA/ARS soybean linkage map [18] based on the probe/enzyme combination used and the number and pattern of the detected fragments.

Detecting polymorphism between J87-233 and 'Hutcheson' with RAPD primers was more successful than with genomic clones. A total of 45 decamer primers were tested to J87-233 and 'Hutcheson', of which 35 were polymorphic.

SCN association

Six RFLPs, 2 RAPDs and 1 SCAR marker showed significance for detecting associations with resistance to SCN races 1, 3, 5 or 14 or multiple races in linear regression analysis (Table 1). No association was found between SCN resistance and either the *t* locus for pubescence color, or the *w1* locus for flower color. Figure 2 shows molecular markers for which associations with SCN resistance have been found in the J87-233 and Hutcheson cross.

subsubsectionLinkage group A2 Most of the RFLP markers for the region of LG A2 spanning ca. 26 cm on either side of the *i* locus (USDA/ARS map) were monomorphic for all five restriction enzymes. However, there was ca. 7.0 cM internal region with three polymorphic markers. The dominant RFLP marker BLT65V described 11.2% of the variation for resistance to SCN race 1, (P=0.0002), and 17.1% for race 3 resistance (P=0.0001) (Table 1). The polymorphism detected by codominant SCAR $548/563_{1100/1025,975}$ was associated with resistance to SCN races 1 and 3

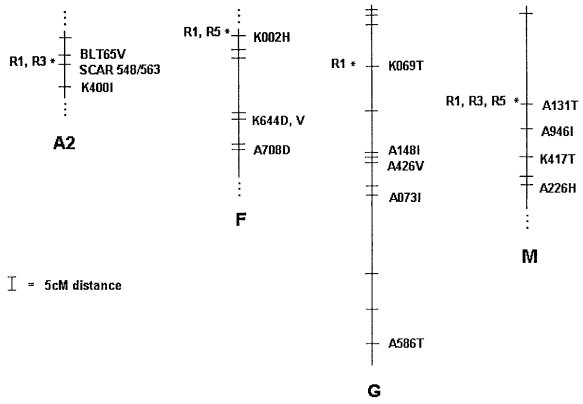

Figure 2. Linkage group regions for QTL for SCN resistance in J87-233. The RFLP marker designation incorporates restriction enzyme for which polymorphism was mapped: I, *Eco*RI; V, *Eco*RV; H, *Hin*dIII; D, *Dra*I; T, *Taq*I. Markers showing significant association with SCN resistance QTL are indicated with asterisks (*). SCN races 1, 3 and 5 are designated by R1, R3 and R5, respectively. SCAR 548/563 segregates for codominant 1100 bp (resistance pattern) and 1025 bp and 975 bp fragments (susceptibility pattern).

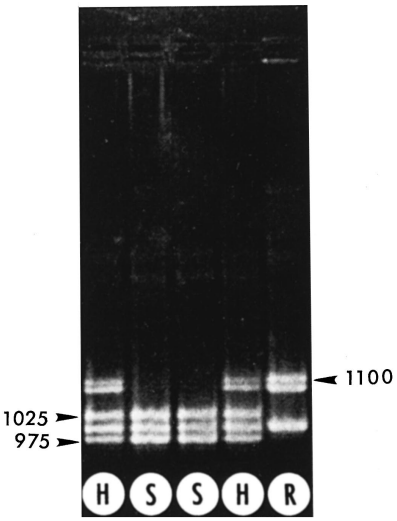

Figure 3. Amplification products of codominant SCAR 548/563 on 2% TBE agarose gel. R, Resistance pattern exhibited by J87-233; S, Susceptibility pattern exhibited by 'Hutcheson'; H, Heterozygous pattern. Polymorphic fragments are indicated by the arrows. Fragment size was estimated with Lambda DNA digested with *Hin*dIII (not shown).

and described 17.5% and 18.9% of the variation, respectively, (P=0.0001). Linkage analysis confirmed that marker BLT65V and SCAR $548/563_{1100/1025,975}$ described the same region of LG A. The resistant, susceptible and heterozygous amplification patterns of SCAR 548/563 are shown in Figure 3.

Another RFLP marker, K400I, was linked to the SCAR $548/563_{1100/1025,975}$ locus at a distance of

Table 1. Molecular marker associations for SCN resistance in the $F_{2:3}$ population of J87-233 × 'Hutcheson'

Marker	Type LG	SCN Race	F value	P≥F	R^2
BLT65V	RFLP A2	1	14.57	0.0002	0.112
		3	23.60	0.0001	0.171
K400I	RFLPA2	1	4.90	0.009	0.078
		3	12.46	0.0001	0.179
$548/563_{1100/1025,975}$	SCARA2	1	11.48	0.0001	0.175
		3	13.01	0.0001	0.189
K002H	RFLP F	1	4.28	0.016	0.072
6		5	3.87	0.024	0.065
K069T	RFLPG	1	6.58	0.002	0.119
		3	2.70	0.072	0.053
A131T	RFLP M	1	6.11	0.015	0.069
		3	6.12	0.015	0.059
		5	5.57	0.020	0.054
A426D	RFLP UL	14	7.74	0.006	0.065
$0A08_{780}$	RAPD UL	14	7.20	0.008	0.059
$0G08_{1130}$	RAPDUL	14	6.03	0.015	0.049

UL, unlinked markers

5.2 cm (Figure 2). K400I was also highly significant for resistance to SCN races 1 and 3 (P=0.009 and 0.0001, respectively). Interval mapping of the BLT65V-SCAR $548/563_{1100/1025,975}$-K400I region by 0.10 cm intervals using MAPMAKER/QTL version 1.1 resulted in the detection of two QTLs for SCN resistance. For race 1, the QTL mapped to the SCAR $548/563_{1100/1025,975}$ with a log-likelihood estimate of 3.3. For race 3, the QTL was localized 1.4 cm from the SCAR $548/563_{1100/1025,975}$ locus toward the K400I locus, with a log-likelihood of 5.6. Pairwise t-tests [16] of IP means between genotypes homozygous and heterozygous for SCAR $548/563_{1100/1025,975}$ showed that the mean IP of individuals homozygous for 'resistance' allele and individuals heterozygous for the SCAR marker were statistically equal (P=0.15 for race 1, and P=0.07 for race 3) indicating dominance at the locus. Mean IP values for individuals homozygous for the 'susceptible' SCAR allele and homozygous for the 'resistance' SCAR allele were significantly different for both races 1 and 3 (P>0.0002).

To further investigate marker-SCN association on LG A, SCAR 548/563 was tested to 50 soybean accessions. Figure 4 shows the UPGMA dendrogram of 12 cultivars and 38 PIs based on response to SCN race 1 and race 3. Two major clusters were identified. One large cluster contained genotypes characterized by the resistance pattern of the SCAR marker and resistance to race 1 and 3, and another cluster contains genotypes with the susceptibility SCAR pattern and susceptibility to race 1 and 3. However, several genotypes did not show this straightforward correlations. For example, PI 92720 exhibited the resistance allele for SCAR 548/563 yet is susceptible to SCN race 1, and 'Columbia' exhibited the susceptible allele for SCAR 548/563 yet is moderately resistant to SCN race 1 and resistant to race 3. PI 209332, used for mapping of SCN resistance genes [5], has a susceptibility molecular pattern for SCAR 548/563, and exhibits moderate susceptibility to SCN race 1 and resistance to race 3. In comparison, PI 88788 exhibits a resistance molecular pattern of SCAR 548/563 and is moderately susceptible to SCN race 1 and resistant to race 3.

Linkage group G

Clones A378, A586, and B053, Bng122 were used to investigate polymorphism in the two opposite regions of LG G. Clones A378, B053 and Bng122 produced monomorphic markers with all five restriction enzymes for J87-233 and 'Hutcheson'. Marker C006 [5], which is located 1.9 cm toward the chromosome end from B053 locus, has not been tested. In the region of major QTL for resistance on LG G [5, 6], no polymorphism for B053 between the primitive resistance sources, and J87-233 and 'Hutcheson' was observed. For clone Bng122, *Dra*I polymorphism was

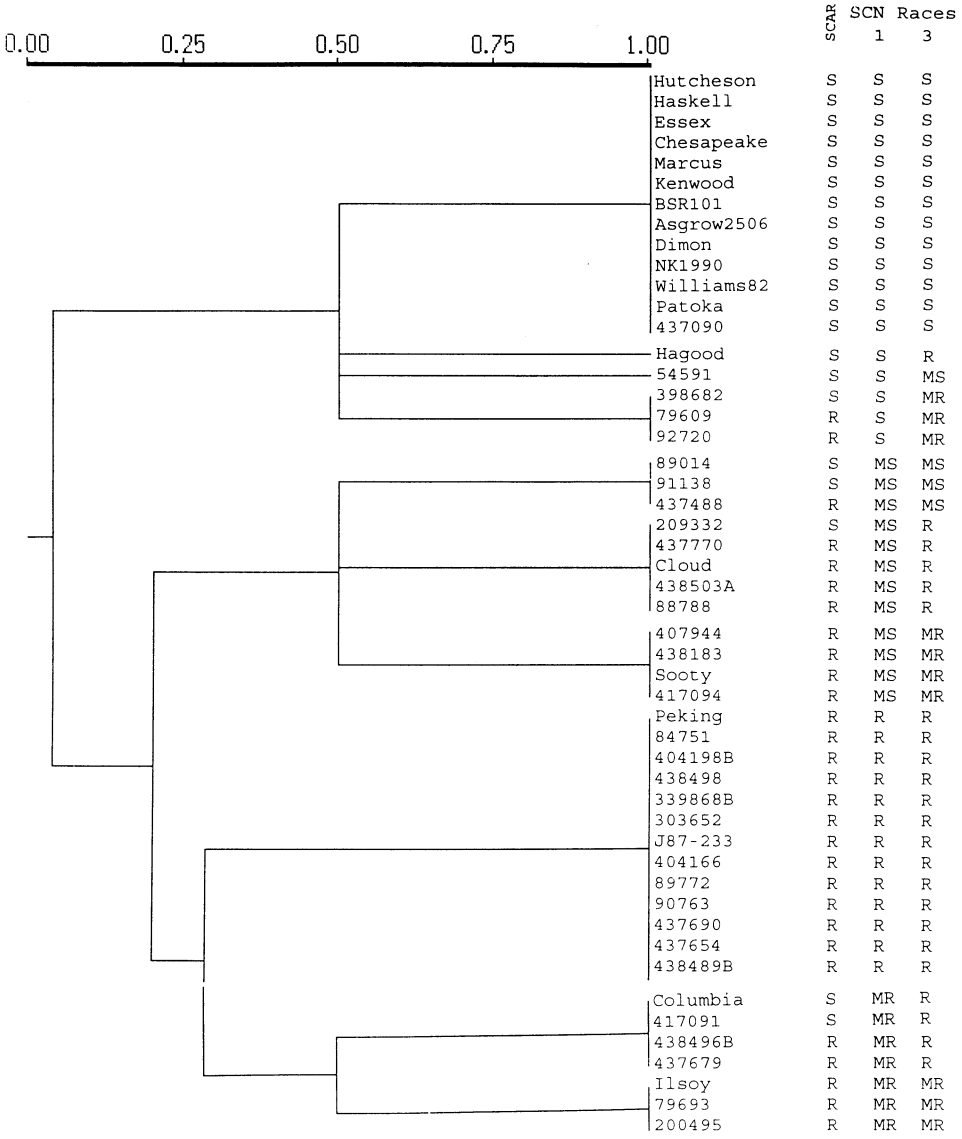

Figure 4. UPGMA consensus tree of 50 soybean accessions developed from SCN race 1 and 3 resistance data. Numeric scale indicates level of similarity between groupings. Genotypes were homozygous for the SCAR 548/563 as confirmed by analysis of 5 individuals per genotype (R, resistance or S, susceptibility SCAR patterns). The SCAR pattern is presented in Figure 3. Response of soybean genotypes are according to Schmitt and Shannon [17]: R, resistant (IP = 0−9%); MR, moderately resistant (IP= 10−30%); MS, moderately susceptible (IP= 31−60%); S, susceptible (IP > 60%).

detected between PI 90763 and two others primitive resistance sources PI 88788 and 'Peking'. Both J87-233 and Hutcheson exhibited the same allelic pattern of PI 88788 and 'Peking'. Marker K69T, c. 18.5 cM from B053-1 [18], was polymorphic, and proved to be significant (P=0.002) for SCN race 1 resistance. Marker K69T described 11.9% of the variation for the race 1 resistance, but there was no significant association with any other race (Table 1). Marker A586T was polymorphic, but not significant for any SCN race resistance.

Linkage groups F, J , M and N

Marker K002H on LG F showed some level of association with race 1 and 5 resistance (P=0.02) (Table 1). About 30 cM of the LG M region were tested with A226, A715, K417, K024,and A131 clones. Only markers A226H and A131T detected polymor-

phisms for this region. Both markers represented the allelic pattern of resistance sources in J87-233. Marker A131T was found to be associated with resistance to races 1, 3 and 5 (P=0.02) and explained significant variation for SCN resistance (Table 1).

For LG J, markers generated by B032, A233, A724 and A199 clones (7.7–15 cM from B032-1 [18]) tested to J87-233 × 'Hutcheson' cross were not significant for any SCN race resistance. A region of LG N, flanked with RFLP markers A280-1 and A071-1 and previously reported by Concibido *et al.* [5], was not significant for SCN resistance.

Vierling *et al.* [20] have reported RFLP markers A06, A567, A487 and A112 of LGs B, S, A and F, respectively, which together described more than 90% of the variation for SCN resistance in a population derived from a cross between cultivars 'Williams 82' and 'Hartwig'. 'Hartwig' was derived from the cross 'Forrest'^{3} × PI 437654. 'Forrest' derives its resistance from 'Peking', which shares similar SCN resistance loci with PI 437654 [21]. In this analysis, the four clones were monomorphic for the J87-233 × Hutcheson cross with the five restriction enzymes tested.

Other associations

Marker A426D was the only RFLP that detected any level of significance for race 14 resistance (P=0.006). The allelic pattern of resistance sources was present in J87-233 for this marker. The two unlinked RAPD markers, $OA08_{780}$ and $OG08_{1130}$, were the only other markers which showed significance for SCN race 14 resistance (P=0.008 and P=0.02, respectively) (Table 1).

Loci interactions

Interactions between detected loci of LGs A, F, G and M (Figure 2) were tested in all possible two-way ANOVA. For race 1, locus BLT65V-SCAR $548/563_{1100/1025,975}$ on LG A2 showed epistatic interaction with markers for SCN resistance on LG F, LG G and LG M at P=0.0001. For race 3, significant interaction was detected between loci on LG A2 and LG M at P=0.0007. No significant loci interaction was detected for race 5.

Discussion

Webb *et al.* [21] as well as Mahalingam and Skorupska [11] have reported a dominant action of the QTL for resistance near the*i* locus conferring seed coat color on LG A. Previously, Matson and Williams [12] showed the existence of a dominant gene, Rhg_4, tightly linked to the *i* locus. J87-233 and 'Hutcheson' carried identical alleles for seed coat color at this locus, hence the population could not be scored for this trait. However, three markers (BLT65V, SCAR $548/563_{1100/1025,975}$ and K400I) close to the *i* locus were informative for this population and also highly significant for resistance to SCN races 1 and 3. The pairwise *t*-tests of IP data also showed dominant QTL action for resistance to SCN races 1 and 3. Retained polymorphisms of LG A2 region near the SCN resistance locus may indicate usefulness of this region in marker-assisted selection for SCN resistance. Indeed, primitive resistance sources and J87-233 had identical marker allele for BLT65V, SCAR $548/563_{1100/1025,975}$ and K400I.

Concibido *et al.* [5, 6] reported no evidence for the presence of a resistance locus on LG A for races 1, 3 and 6, for populations derived from PI 88788, PI 90763, 'Peking' and PI 209332. Differences in the QTL detection between research groups are likely due to variability of germplasm as well as diversity of SCN isolates used for bioassays. This is supported by Skorupska *et al.* [19] reporting molecular diversity and SCN response in primitive germplasm-labeled 'Peking'. The combination of SCAR $548/563_{1100}$ resistance allelic marker and black seed coat color in tested soybean genotypes suggests that it may represent ancestral gene arrangement. In support of this is a documented linkage of the SCN resistance gene and the *i* allele for seed coat color of resistant plant introductions and the presence of the *i* allele in soybean progenitors. Possible recombination between the SCAR allele and the QTL encoding resistance (or mutations) in the region on LG A2 could explain the lack of a straightforward correlation between the SCAR pattern and SCN response in the tested soybean accessions. The observed variation may also imply that the locus for resistance may be quite complex with closely linked QTLs encoding specific responses to different races. The SCN resistance locus might be a cluster of genes, within which new resistance genes can occur through DNA rearrangements, i.e., unequal crossing over or mutation. We don't know if the detected separate QTLs on LG A2 are the result of statistical artifact or indeed are two closely linked genes. Positional cloning in this region should resolve uncertainties regarding organization of the locus for SCN resistance on LG A2.

Concibido *et al.* [6] reported a major locus for SCN resistance on LG G. In our study, the markers in this region were uninformative and only marker K69T, which is 18.5 cm from the reported QTL [18] was significant for race 1. Since major QTL could be detected by markers 10–15 cm from QTL peak [4], the strong association of the K69T with SCN resistance exemplifies the importance of the previously identified locus on LG G. Recombination within this region may have occurred resulting in the loss of the Bng122 and B053 resistance marker allele while the resistance phenotype was retained in the J87-233. Interestingly, only PI 90763 had a different allele for Bng 122, and despite the use of this germplasm later in development of J87-233, the allele was not transmitted. It has been suggested that the region of LG G harbors alleles for yield suppression [5]. Due to this possibility, polymorphism of Bng122 and B53 might have been eliminated along with alleles for yield suppression in the development of J87-233. Further, the distal location of the QTL as indicated by its map position on the LG G [4, 5] may contribute to its frequent recombination. The expansion of the linkage map resulting from higher recombination frequency in the terminal chromosomal region may cause a drag of the QTL effect along large genetic distance as was observed in the J87-233 × 'Hutcheson' population.

Several investigators [4, 8, 11, 21] previously reported evidence of epistatic interactions amongst loci on LGs A2, G and M involving SCN race 3 resistance. Similar analyses in J87-233 × 'Hutcheson' population indicate an epistatic relationship between LG A2 and G, but only for race 1. This would further support the previous evidence that epistatic interactions between loci of LG A2 and G play a significant role in the expression of soybean resistance to SCN. The individual marker on LG G, K69T, had been found to be significant for race 1 resistance and not for race 3. Interaction of LG G with LG F and M loci was also shown for race 1 in the J87-233 × 'Hutcheson' population. Further, since contribution of PI 90763 to resistance in J87-233 was relatively recent, the observed interactions maybe extended to PI 90763 rather than 'Peking' or PI 88788.

The use of cross J87-233 × 'Hutcheson' has several advantages for assessing the applicability of molecular markers for QTL detection and their eventual use in breeding. The population is a F_2 intercross. Any F_2 population between two highly inbred lines of different origin, as in the case of J87-233 and Hutcheson, represents the greatest amount of diversity and variability that can be generated at any locus with that cross. Using this type of cross for genetic mapping research can best accomplished with a large sized population. We employed a modest population of 125 F_2 lines for which eight linkage groups, covering ca. 30% of the genome, were tested and some significant associations might have not been uncovered; for example, none of the tested genomic region was associated with race 2 SCN in this population. Highly significant marker associations with SCN resistance QTL are infrequent in an F_2 population compared to populations comprised of RILs in which moderate phenotypes may be lost and extreme phenotypes begin to dominate. Detecting marker associations at highly significant levels in the hybrid population between advanced breeding germplasm and cultivar, where differences in genetic noise have not been reduced as with recombinant inbred lines (RILs) is exceptional, and lends credence to their legitimacy. In our study, the confidence level with which two major loci have been detected reduces the probability of Type I errors in which marker associations are detected that are actually false or less significant. Our findings, and the corresponding genomic regions identified by Chang *et al.* [4] in a 100 RIL population derived from SCN-resistant 'Forrest' and SCN-susceptible 'Essex', suggest that the regions on LG A2 and LG g are of the greatest utility for use in breeding populations. However, the detected associations between molecular markers and SCN resistance are yet unreliable in revealing all recombinational events occurring in breeding programs for SCN resistance.

Acknowledgements

This paper is technical contribution 4300 of the South Carolina Agricultural Experiment Station. The research was supported by the South Carolina Farm Bureau, South Carolina Soybean Promotion Board and The United Soybean Board.

References

1. Arelli P.R, Wilcox JR: Soybean germplasm resistant to races 1 and 2 of *Heterodera glycines*. Soybean Genet Newsl 23: 186–191 (1996).
2. Bendich AG, Rogers SO: Extraction of DNA from milligram amount of fresh, herbarium and mummified plant tissue. Plant Mol Biol 5: 69–76 (1995).
3. Buss GR, Camper Jr HM, Roane CW: Registration of 'Hutcheson' soybean. Crop Sci 28: 1024 (1988).

4. Chang SJC, Doubler TW, Kilo VY, Abu-Thredeih J, Prabhu R, Freire V, Suttner R, Klein J, Schmidt ME, Gibson PT, Lightfoot DA: Association of loci underlying field resistance to soybean sudden death syndrome (SDS) and cyst nematode (SCN) race 3. Crop Sci 37: 965–971 (1997).
5. Concibido VC, Denny RL, LangeDA, Orf JH, Young ND: RFLP mapping and marker-assisted selection of soybean cyst nematode resistance in PI 209332. Crop Sci 36: 1643–1650 (1997).
6. Concibido VC, Lange DA, Denny RL, Orf RL, Young ND: Genome mapping of soybean cyst nematode resistance genes in 'Peking', PI 90763, and PI 88788 using DNA markers. Crop Sci 37: 258–264 (1997).
7. Keim P, Olson TC, Shoemaker RC: A rapid protocol for isolating soybean DNA. Soybean Genet Newsl 18: 150–152 (1988).
8. Kilo VY, Abu-Thredeih J, Suttner B, Chang SJC, Gibson PT, Lightfoot DA: Coinheritance of resistance to SCN and SDS in Pyramid × Douglas. Soybean Genet Newsl 24: 126–127 (1997).
9. Lincoln S, Daly M, Lander E: Constructing genetic maps with MAPMAKER/EXP 3.0, 3rd ed. Whitehead Institute Technical Report (1992).
10. Lincoln S, Daly M, Lander E: Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1, 2nd ed. Whitehead Institute Technical Report (1992).
11. Mahalingam R, Skorupska HT: DNA markers for resistance to *Heterodera glycines* I. race 3 in soybean cultivar Peking. Breed Sci 45: 435–443 (1995).
12. Matson AL, Williams LF: Evidence of a fourth gene for resistance to the soybean cyst nematode. Crop Sci 5: 477 (1965).
13. Rao-Arelli AP, Anand SC: Genetic relationships among soybean plant introductions for resistance to race 3 of soybean cyst nematode. Crop Sci 28: 650–652 (1988).
14. Riggs RD, Schmitt DP: Complete characterization of the race scheme for *Heterodera glycines*. J Nematol 20: 392–395 (1988).
15. Rohlf JF: NTSYS-pc numerical taxonomy and multivariate analysis system, version 1.80. Applied Biostatistics, New York (1994).
16. SAS: SAS/STAT User's Guide, version 6.0, 3rd ed. SAS Institute, Cary, NC (1990).
17. Schmitt DP, Shannon G: Differentiating soybean responses to *Heterodera glycines* races. Crop Sci 32: 275–277 (1992)
18. Shoemaker RC, Specht JE: Integration of the molecular and classical genetic linkage groups. Crop Sci 35: 436–446 (1995).
19. Skorupska HT, Choi IS, Rao-Arelli AP, Bridges WC: Resistance to soybean cyst nematode and molecular polymorphism in various sources of Peking soybean. Euphytica 75: 63–70 (1994).
20. Vierling RA, Faghihi J, Ferris VR: Association of RFLP markers with loci conferring broad-based resistance to the soybean cyst nematode (*Heterodera glycines*). Theor Appl Genet 92: 83–86 (1996).
21. Webb DM, Baltazar BM, Rao-Arelli AP, Schupp J, Clayton K, Keim P, Beavis WD: Genetic mapping of soybean cyst nematode race-3 resistance loci in the soybean PI 437.654. Theor Appl Genet 91: 574–581 (1995).
22. Weisemann JM, Matthews BF, Devine TF: Molecular markers located proximal to the soybean cyst nematode resistance gene, Rhg_4. Theor Appl Genet 85: 136–138 (1992).
23. Young LD: Registration of J87-233 soybean germplasm with resistance to soybean cyst nematode races 1, 2, 3, and 5. Crop Sci 33: 348 (1993).